SURFACE MODIFICATION FOR BIOCOMPATIBILITY

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Figure. 1. Neurons dissociated from embryonic day 14 cerebral cortex cultured in serum-free medium on DETA/ 13FMe_2Cl patterns after 24 hrs. Phase-contrast microphotographs showing examples of high compliance of neural soma and their processes to DETA lines (versus 13F spaces). A.B. Overview of cultures of cortical cells plated onto a line/space-patterned substrate consisting of alternating parallel stripes of DETA and 13F (A. 10 (13F) x 5 (DETA) μm and B. 32 x 10 μm). C. Higher magnification of cortical cells on a line/space pattern 10 (13F) x 10 μm (DETA).

Figure. 2. Neurons dissociated from embryonic day 14 cerebral cortex and cultured in serum-free medium on DETA/ 13FMe_2Cl patterns for 4 days. Phase-contrast microphotographs show less compliance of neural soma to lines than at 24 hrs. Cell bodies (but not processes) lose pattern fidelity on fine line patterns (C. 5 x 3 μm), but are more faithful on patterns with wider features (A. 22.5 x 10 μm; B. 10 x 5 μm).

PROJECT SUMMARY

The purpose of this project is to develop modified artificial surfaces for implantable biocompatible electrodes in the cerebral cortex. To this end, primary objectives of the project have been focused on the development of a defined *in vitro* cell culture model and to use *in vitro* cultures to select surfaces that could be used to control the interaction of neurons, astrocytes, and/or microglia in embryonic and adult stages of development. In Quarterly report #9, we summarized our work in which we showed that:

1) we had established an *in vitro* system that mimics the environment found in CSF and studied cortical neurons, astrocytes and microglia in this system.
2) embryonic stem cells (E12) exhibited adult markers in our *in vitro* system (after 7-14 days) and compared these results to adult cells in the same system.
3) surface analysis was a key component in understanding the results, and provided a quantitative measure of system performance.
4) surface composition could be used to select for neuronal phenotype.

Much of our subsequent effort this quarter has been spent on finalizing these studies and writing manuscripts for publication (pg. 5). These data form the basis for the selection of candidate SAMs for modification of biocompatible implantable electrodes for *in vivo* work to be started this coming quarter. This Quarterly report will address some preliminary *in vitro* studies with patterning of cortical cells on SAM substrates and discuss application toward the proposed *in vivo* research.

Overall project objectives

a) Selecting candidate surfaces that are likely to enhance the microscopic mechanical stabilization of a microstructure implanted within the central nervous system/
b) Selecting candidate organic surfaces that are likely to enhance the close approximation of neurons or neuronal processes to specific regions of implanted silicon microstructures;
c) Developing or adapting available methods to bond the selected organic molecules to a silicon dioxide surface like the surface of a micromachined electrode (Tanghe and Wise: A 16-channel CMOS neuronal stimulating array (*IEEE Trans. Sol State Circuits* 27: 69-75, 1992) and to chemically characterize these surfaces before and after protein adsorption.

1. The attachment method shall be stable in saline at 37'C for at least 3 months;
2. To use silane coupling as the method of attachment;
3. To use the silanes to control the spatial extent (ie., the pattern) of the deposited surface.

d) Developing a cell culture or other suitable model of mammalian cortex and investigate the growth and adhesion of neurons, glia, micro-glia, and other cells present in the nervous system on substrates coated with the selected surfaces;
e) Cooperating with other investigators in the Neural Prosthesis Program by coating microelectrodes (estimated 50 over the contract period with the most promising materials for *in vivo* evaluation as directed by the NINDS Project Officer.

BACKGROUND

Biomaterials that penetrate into the central nervous system as the microscopic electrode shafts of neural prostheses interact with neural and other tissues on a cellular and molecular level. In order to achieve tight coupling between these implanted microelectrodes and the target neural substrate, this interaction must be understood and controlled. Controlling the interaction requires an understanding of how cells, including neurons and glia, and extracellular proteins respond to the surface chemistry and any leachable substances of implanted biomaterials. This contract supported research will study these interactions with a long-term goal of rationally designing microelectrode surfaces to promote specific tissue interactions.

Presently, available clinical neural prosthetic implants typically use stimulus levels that excite volumes of neural tissue ranging from cubic millimeters to cubic centimeters around the electrode. Because of the large stimulus intensities required, precise control of the response of neurons within the first few cell layers of an implanted electrode has not been necessary. Recent developments in the areas of micromachining and fabrication of silicon integrated circuit microelectrodes have introduced the possibility of controlled stimulation of smaller volumes of neural tissue--on the order of one thousand to one hundred thousand times smaller than those used today.

The efficiency of these microelectrodes depends on the micro-environment around stimulating sites. The surface of the microelectrodes and the proteins that adsorb to this surface have a major impact on the way in which different cell populations react to the implant. Neural growth cones are sent out from many neurons around a microelectrode following implantation. With appropriate surfaces, it may be possible to get selected neurons to send processes directly to the microelectrodes. Glia and other cells also respond to an implanted electrode. With appropriate surfaces it may be possible to promote cell adhesion and anchoring of some areas of the implant structure while leaving other areas with minimal response from glial cells. This study will investigate cellular and molecular responses to specific surface modifications of silicon microelectrodes.

METHODS

Self-Assembled Monolayers

To prepare biocompatible surfaces for neuronal cell survival and axonal outgrowth we modified different materials with self-assembled monolayers (SAMs). Three of the substrate materials that have been modified are silicon, glass and silicon nitride. These materials are cleaned prior to modification to ensure the removal of surface contaminants and to create reactive silanol groups on the substrates surface. These surface silanol or hydroxyl groups are used as a final reactive site for preparing SAMs (for detailed reference Quarterly Report #9). The SAMs provide an effectively new surface whose chemical properties may be controlled by careful choice of silanes containing selective chemical functional groups.

Table 1

Abbreviation	Chemical	Contact angle, θ_{adv}
OTS	octadecyltrichlorosilane	103
NDEC	N-decyltrichlorosilane	98
13F	(tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-dimethylchlorosilane	90
TCMD	(10-Carbomethoxydecyl) dimethylchlorosilane	67
PPDM	(3-phenylpropyl)dimethylochlorosilane	64
CP	3-cyanopropyldimethlchlorosilane	63
TP	triphenylchlorosilane	60
PEDA	(aminoethylaminomethyl)phenethyltrimethoxysilane	56
MTS	3-mercaptopropyltrimethoxysilane	54
MTS-OX	oxidized 3-mercaptopropyl-trimethoxysilane	46
NBUT	n-butyldimethylchlorosilane	44
APTS	aminopropyltrimethoxysilane	42
DETA	trimethoxysilylpropyldiethylenetriamine	37
EDA	N-(2-aminoethyl)-3-aminopropyltrimethoxysilane	35
MAP	N-methylaminopropyltrimethoxysilane	34
D-MAP	(N,N dimethyl-3-aminopropyl)trimethoxysilane	31
PEG-350	triethoxysilyl polyethyleneglycol (mw = 350)	30
UAD	11- undecanoic acid dimethylsiloxy	21

The matrix of SAMs that have been examined for cell culture survival and axonal outgrowth are shown in Table 1. The average water advancing contact angles are given for each monolayer prepared in descending order starting with the most hydrophobic surface.

The contact angle measurement is a good representation of the hydrophobicity of the surface. OTS is the most hydrophobic surface shown with an average contact angle of

103 degrees. Neuronal survival is not only based on some critical amount of hydrophobicity but also on the functional groups presented by each monolayer.

We chose to utilize two substrates for our *in vitro* pattern studies, one hydrophilic SAM, DETA, proven to be good for cell attachment and survival, and a substrate, $13FMe_2Cl$, to which cells poorly adhere.

Pattern Preparation

Coverslips were cleaned, dried and placed in a solution of DETA (toluene) and dried. Contact angles measured were: advancing angle (43') and receeding angle (15').

Line/space patterns were prepared using an ArF excimer laser. The DETA treated coverslips were placed in contact with the quartz/chromium mask by sandwiching the slip between the mask and a piece of glass. The irradiated area consisted of 21 regions of alternating chromium lines and quartz spaces of varying feature size.

The slips were backfilled with a solution of $13FMe_2Cl$. The advancing and receeding contact angles for $13FMe_2Cl$ were both, 83'.

Cell were plated, as described previously, (Quarterly reports 1-9, Shaffner, et al.1996) in defined medium. Briefly, Cortical cells were enzymatically dissociated from embryonic day 14 rat cerebral cortices and plated onto patterned glass coverslips at a density of 125 K per coverslips. Cultures were maintained in serum-free medium (MEM + N3/g) in 10% CO2 incubator.

RESULTS

We established a method for patterning cortical neurons at low densities to observe the fidelity of individual cells and their processes . Results showed that at Day 1 cell bodies and processes were highly faithful to line/ space patterns, where they mostly adhered to the DETA lines. By Day 4, cell bodies often spanned 13F spaces to adhere on adjacent DETA lines, but their processes were, to some degree, still faithful to DETA lines on the patterns. These results indicate that SAM substrates offer the promise of controlled positioning and guidance of growing neurites. The next step will be to examine these phenomena *in vivo.*

PROPOSED *IN VIVO* STUDIES

A meeting on July 18th with the NIH program project manager has led us to focus on *in vivo* study of surface-modified electrodes for the remainder of the contract period (August to December 1997). We will modify an electrode surfaces with self-assembled monolayers (SAMs) and, possibly, with biological macromolecules. The biocompatibity of these stimulating electrodes will be evaluated after implantation in the rat cerebral cortex.

The animal surgery and post-operational care will occur at Uniform Services University of the Health Sciences, Bethesda, with Dr. Sharon Juliano. Tissue immunocytochemistry and analysis will be carried out at SAIC, Biotech Applications Division, Taft Ct. We can start experiments in the middle of August.

Scope of proposed experiments

The goal of this contract is to design interfaces to examine and control the interaction of surfaces with biological systems both *in vitro* and, ultimately, *in vivo*. We have used self-assembled monolayers, in conjunction with biological macromolecules, to coat electrode materials to render them biocompatible for cell culture. However, it is not clear whether these newly-designed interfaces will control the interaction of the surfaces with neural cells in the cerebral cortex. Therefore, we will proceed with the following *in vivo* experiments as they were originally planned:

We will implant surface-modified iridium electrodes in the parietal cortex of rats. About 24 adult Sprague-Dawley rats are needed. Surgery will be performed by Wu who is experienced in animal brain surgery. Postsurgical care will be provided by SAIC staff in USHUS animal facility. Rats are observed twice daily during the week and once daily on weekends to ensure uneventful recovery from anesthesia and surgery; to administer supportive fluids, analgesics, and other drugs as required; to provide adequate care for surgical incisions; to empty the animal's bladder by manual compression 2 times a day for 7-10 days; and to maintain appropriate medical records. Animals will be housed individually for 12 hours, 1, 7, 14 days and 1, 2, 3 and 4 months respectively before sacrifice. The rats will be killed quickly and humanely by overdose with sodium pentobarbital (80 mg/kg). The brain will be fixed with 4% paraformaldehyde in 0.1 M phosphate buffer by vascular perfusion via the left heart ventricle in a perfusion hood.

Brain sections will be cut serially in 12-20 μm on a cryostat, thaw-mounted onto gelatin-coated slides. Implant surface characterization using morphology, XPS, immunostaining will occur at SAIC, Taft Ct. We will examine the following:

1) Histological and cell- phenotypic characteristics

2) Neuronal responses to surface-modified implants

3) Glial responses to surface-modified implants

4) Extracellular proteins---Immunolocalization of macromolecules surrounding implants

5) Cytokines released by astrocytes, microglia, microphages and neurons before and after implantation

PAPERS IN PREPARATION

Hickman, James J., Coulombe, Margaret G., Ma, Wu, and Rama Sathanoori. Development of an *In Vitro* system to Study Neuronal Development and Regeneration of Axonal Processes of Explanted Adult Cortical Neurons.

Hickman, James J., Coulombe, Margaret G., and Wu Ma. Differential Response of Astrocytes to Surfaces, bFGF and Implications for Implants.

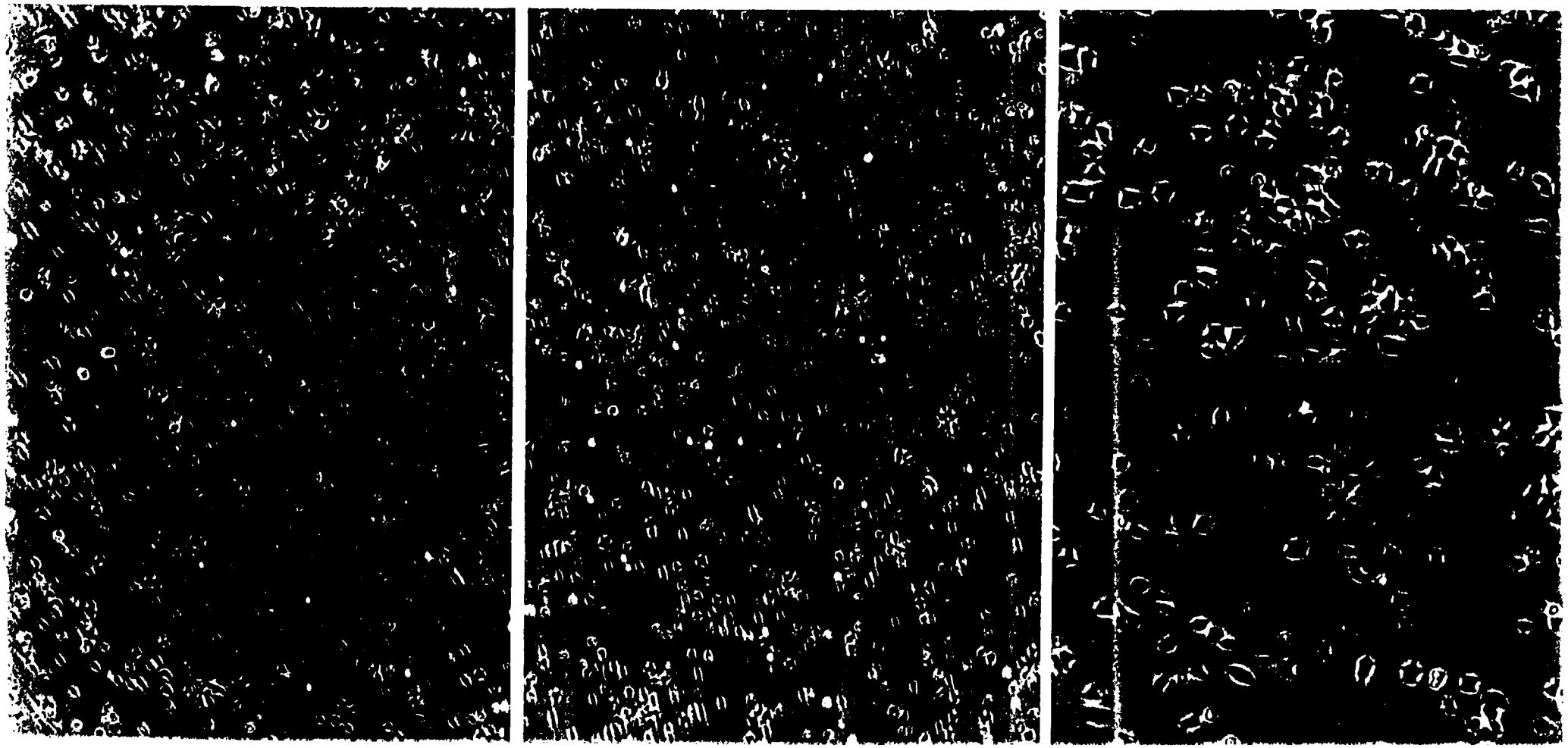
Hickman, James. J., Jung, David R., and Margaret Coulombe. Correlation of Cortical Neuronal Morphology to XPS Analysis of Substrate Materials.

Jung, D. R., Coulombe, M. G., Bateman, K. F., Sathanoori, R. S., Shaffner, A. E., Barker, J. L, Stenger, D. A, and J. J. Hickman. XPS analysis of protein layers deposited by in vitro neuronal cell cultures.

Ma, W., Coulombe, M. G., Jung, D. Sathanoori, R., and J. J. Hickman. Selective Expression of GABAergic and Glutamatergic Neurons on Organosilane Surfaces.

Compliance of cortical neurons to patterned SAMs (DETA/13F)

E14 cells in serum-free medium for 1 day



A

10 (13F) x 5 (DETA) μm

B

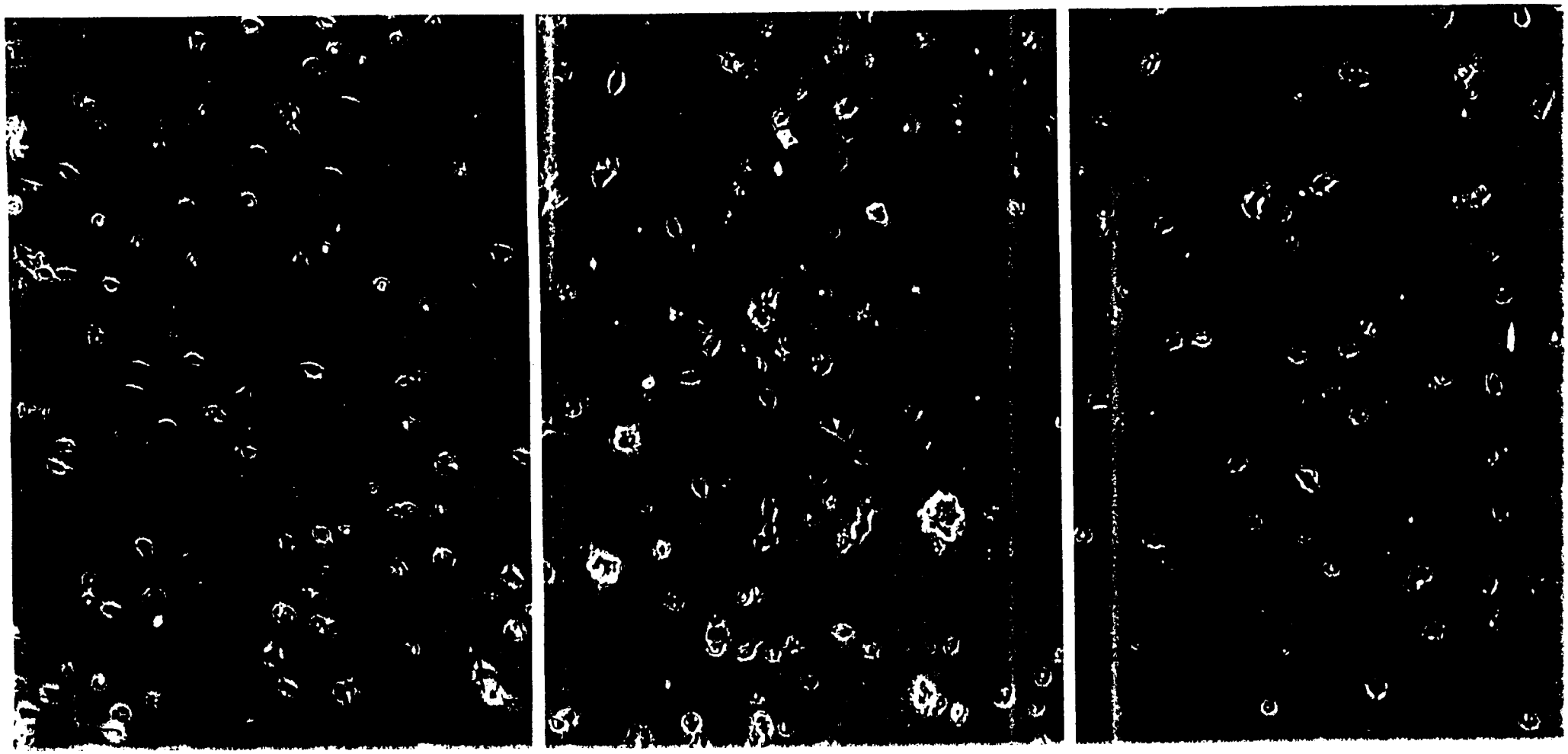
32 (13F) x 10 (DETA) μm

C

10 (13F) x 10 (DETA) μm

Compliance of cortical neurons to patterned SAMs (DETA/13F)

E14 cells in serum-free medium for 4 days



A

22.5 (13F) x 10 (DETA) µm

B

10 (13F) x 5 (DETA) µm

C

5 (13F) x 3 (DETA) µm